Novel method to characterize paramagnetic contrast agents association to 2-dimensional surfaces

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Introduction

Receptor mapping of cells lining blood vessels is one of the major applications of molecular MR imaging using targeted contrast agents, for example for atherosclerosis or angiogenesis characterization. In these situations, the contrast agent may bind to or accumulate in a very thin layer of cells. It is not straightforward to predict how the enhanced water longitudinal relaxation rate at this surface affects the relaxation rate of water in a typical voxel much larger than the dimensions of the cells containing the contrast agent. Determination of the relaxation rate as function from the distance to a surface bound contrast agent will yield valuable information on the limits of MRI-based detection of contrast agents.

Aim

The goal of this study was to develop a novel method to determine the surface relaxation rate (ρ) of a surface bound paramagnetic contrast agent.

Materials and methods

Preparation of the surface: As a model surface we have used a thin layer of collagen fibers to which CNA35-functionalized paramagnetic liposomes were bound. CNA35 is an adhesion protein that binds specifically to collagen. The surface was created on a clear flatbottom Costar 8-well strip-plate. The wells were incubated overnight at 4 °C with 50 μL of 55 μg/mL rat-tail collagen type I in a solution of 20 mM HEPES and 135 mM NaCl (pH 7.4). The non-bound collagen was then aspirated from the wells using an automated Wellwash AC plate washer and the wells were rinsed 3 times with 300 µL HBS at 20 °C. The wells were then blocked with 250 μL of 5% (w/v) milk powder in HBS for 2 h at 20° C, aspirated and rinsed 5 times with 300 μL HBS. 50 µL of a solution of either paramagnetic CNA-35-functionalized or non-functionalized liposomes in HBS or just plain HBS was added to each well and incubated 3 h at 20 °C. The paramagnetic liposomes (Figure 1) containing Gd-DTPA-bisstearyl amine (Gd-BSA), DSPC, cholesterol, PEG2000-DSPE, and Mal-PEG2000-DSPE were prepared by lipid film hydration [1]. The typical diameter of the liposomes is in the order of 200 nm. CNA-35 obtained as described previously by Krahn et al. [2] was coupled to the liposomes by a sulfhydryl-maleimide coupling method [1]. Uncoupled CNA-35 was separated from CNA-functionalized liposomes by ultracentrifugation. After incubation, the wells were aspirated and washed 10 times with 300 μL HBS.

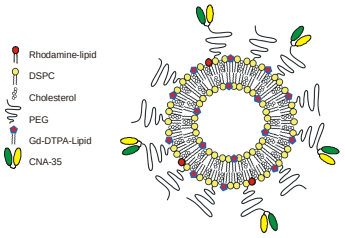

Figure 1: Cartoon of paramagnetic CNA35-functionalized liposome

Measurement method: The measurements were performed at room temperature with a micro imaging NMR setup based on the so-called GARField method [3]. The set-up consists of an electromagnet with specially shaped pole tips, generating a magnetic field of 1.4 T with a gradient of 36.4 T/m perpendicular to the coating film [4]. The saturation recovery sequence was used to measure the restoration of the magnetization, characterized by the T_1-relaxation time. The saturation recovery sequence used in our experiments consists of 4 saturation pulses with an average pulse length of 0.7 μs, corresponding to an average flip angle of 70°, spaced with an average time of *ts* = 1 ms. The saturation recovery sequence is given by $(\tau_s\text{-}70^o)_4\text{-}\tau_r\text{-}90^o\text{-}\tau_e\text{-}\ 90^o\text{-}\tau_e\text{-}echo$, in which τ_s, τ_r, τ_e are the time between the saturation pulses, time to allow the magnetization to recover, and 2 τ_e the echo time, respectively. The echo time 2 τ_e is set at 200 μs, giving a spatial resolution of 5 μm in our setup. For each recovery time τ_r, varying in 20 steps between 0 and 10 s, 4096 echoes were acquired.

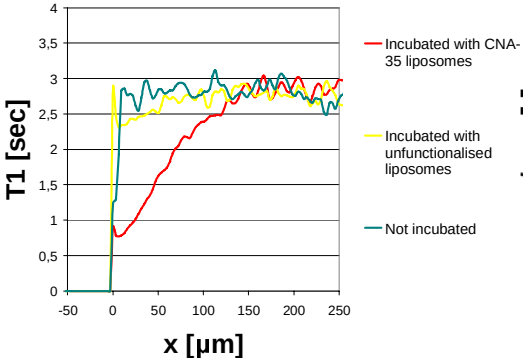

Figure 2: T1 as function of the distance to the surface (0 represents the bottom of the plate)

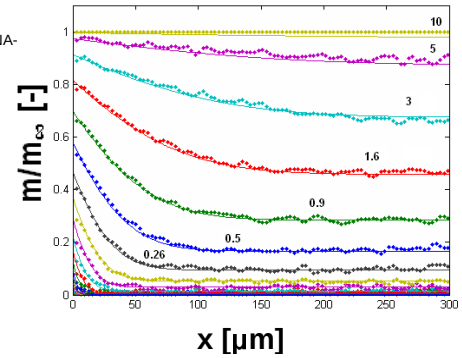

Figure 3: Experimental data (points) and theoretical fit (solid lines) from 20 different inversion times (numbers)

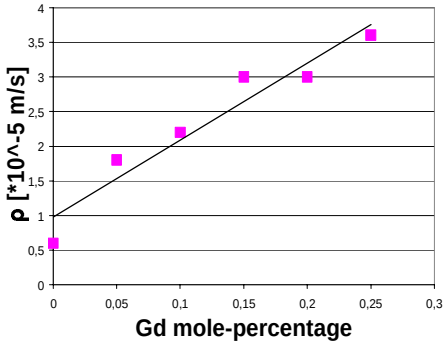

Figure 4: Surface relaxation rate as function of increasing gadolinium mole-percentage in liposomes. A linear trend is displayed

Results

Figure 2 shows the T_1 as function of the distance to the surface. A reduction in T_1 near the surface was observed for the CNA35-liposome incubated well, which extents up to about 120 µm from the surface. In contrast, for the wells not incubated or incubated with non-functionalized liposomes no or very limited reduction was observed. In figure 3 the measurements for the CNA35-liposomes are presented in a different fashion. The normalized magnetization m/m_∞ is plotted as function of the distance to the surface, resulting in 20 lines for the different inversion times. This allows the data to be described with the following equation:

$$\frac{m}{m_\infty} = 1 - \exp\left(-t/T_{1,b}\right)\left[erf\left(\frac{x}{\sqrt{4Dt}}\right) + \exp\left(\frac{\rho[x+\rho t]}{D}\right)erfc\left(\frac{x+2\rho t}{\sqrt{4Dt}}\right)\right]$$

Here $D=2.1\pm0.1\cdot10^{-9}$ m^2/s is the diffusion of water, $T_{1,b} = 2.65 \pm 0.1$ s is the bulk longitudinal relaxation time of water, and t is the inversion time. The surface relaxation rate ρ (units m/s) is the only variable, which allows for accurate fitting of the curves presented in figure 2. A simultaneous fit with the above equation to all the curves resulted in $\rho = 4.0 \pm 0.5\cdot10^{-5}$ m/s for this example. Finally figure 4 presents the dependence of ρ on the mole-percentage of Gd-BSA incorporated in the liposomes, illustrating that the surface relaxivity scales linearly with the amount of contrast agent present at the surface.

Conclusions

In this study it was shown that high-resolution NMR depth profiling by using a saturation recovery pulse sequence is capable of determining the surface water longitudinal relaxation rate induced by a surface-bound contrast agent. This method will be helpful in studying the behavior of targeted contrast agents at biological interfaces, which are presently developed in the area of molecular imaging. With high resolution NMR depth profiling well controlled in vitro studies can be performed in order to gain insight in the role of factors like diffusivity, flow, Gd-concentration, binding characteristics and the subcellular distribution of contrast agents.

[1] Mulder et al. Bioconjug. Chem. 2004: 15: 799-806, [2] Krahn et al. Anal. Biochem. 2006: 350: 177-185, [3] Glover et. al. J. Magn. Reson. 1999: 139: 90-97, [4] Erich et al. Prog. Org. Coat. 2005: 52: 210-216;